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Astragali Radix elicits anti-inflammation *via* activation of MKP-1, concomitant with attenuation of p38 and Erk

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Abstract

Although Astragali Radix (Astragalus, AR), the root of *Astragalus membranaceus* (Fisch) Bunge, is widely used in oriental medicine for tonifying the immune response and improving circulation, the underlying mechanism(s) by which these effects are induced remains unclear. Here, we report that AR displays anti-inflammatory effects in zymosan air–pouch mice by reducing the expression of iNOS, COX-2, IL-6, IL-1β and TNF-α and by decreasing the production of nitric oxide (NO). In a similar manner, AR reduces the expression of IL-6, iNOS, and COX-2 in lipopolysaccharide (LPS)-treated Raw 264.7 cells. We further demonstrate that AR attenuates the activity of p38 and Erk1/2 and stimulates mitogen-activated protein kinase phosphatase-1 (MKP-1) in LPS-treated Raw 264.7 cells. Additionally, AR interferes with the translocation of NFκB to the nucleus, subsequently resulting in NFκB-dependent transcriptional repression. Taken together, these data reveal that AR has an anti-inflammatory effect that is mediated by the MKP-1-dependent inactivation of p38 and Erk1/2 and inhibition of NFκB-mediated transcription. These results imply that the AR herb has a potential anti-inflammatory activity.

Keywords: Astragali Radix; Anti-inflammation; p38; Erk1/2; Mitogen-activated protein kinase phosphatase-1; NFκB

1. Introduction

The search for naturally occurring anti-inflammatory compounds and anti-cancer drugs that may be effective for the treatment of many human diseases is currently a worldwide pursuit. One of the most popular herbs that have been used for thousands of years to treat a variety of ailments in oriental medicine is Astragali Radix (AR; Astragalus, *Hwang Gi* in Korean), which is the root of *Astragalus membranaceus* (Fisch) Bunge (Fabaceae family). Traditionally, AR was used to treat weakness, wounds, anemia, fever, multiple allergies, chronic fatigue, loss of appetite, uterine bleeding, and uterine prolapse (Kim et al., 2003). Medicinally, AR is used as a diuretic and tonic herb in many Asian countries, to enhance physical strength and endurance, strengthen the immune system, lower blood pressure, and promote excretion and circulation (Zhao et al., 1990; Sheng et al., 2001; Yu et al., 2006). Clinically, AR is used to treat chronic phlegmatic disorders and general gastrointestinal disturbances including stomach ulcers and diarrhea (Yang, 1993; Hei et al., 2005).

The mechanism by which AR mediates the above-mentioned effects is unclear. Studies on the use of AR in various human diseases have generated evidence suggesting that this herb may act as an immuno-regulator that can enhance strength,

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immunity, and circulation. Reports also state that AR astragalosides may inhibit liver fibrosis by reducing TNF-α and TGF-β1 production, as well as by scavenging active oxidants in activated Kupffer cells (Gui et al., 2006). In immuno-suppressed mouse spleen cells and Raw 264.7 cells, AR has been shown to induce proliferation (Lee et al., 2003, 2005) and to reverse not only cyclophosphamide-induced repression of NK cell activity (Jin et al., 1994) but also impaired cell-mediated immunity in burned mice (Liang et al., 1994). AR also appears to exert immunomodulatory effects by regulating the expression of cytokines such as IL-1α, IL-1β, IL-6, and inducible nitric oxide synthase (iNOS) as well as the production of nitric oxide (NO) (Lee et al., 2005).

The mitogen-activated protein (MAP) kinase signaling cascade (Dong et al., 2002) plays a critical role in innate immune responses. In response to inflammatory signals including microbial or viral infection, UV-irradiation, and burning, the MAP kinase cascade is activated through phosphorylation of p38, Erk1/2, and JNK, together with activation of NFκB (Sumbayev and Yasinska, 2006). This MAP kinase activation is terminated, and returns to base levels within several hours, following its stimulation (Pouyssegur et al., 2002). Factors that negatively regulate MAP kinase activity, by binding to and dephosphorylating active MAP kinases, form part of the dual specificity MAP kinase phosphatase (MKP) family. Consistent with this notion, several recent investigations have shown that MAP kinase specific phosphatase-1 (MKP-1) deficiency results in enhanced and prolonged p38 and JNK activation (Nimah et al., 2005; Wu and Bennett, 2005; Zhao et al., 2005). MKPs were initially identified as early response genes (Lau and Nathans, 1985; Sun et al., 1993) and are expressed ubiquitously in response to growth factors, stress, or heat shock (Farooq and Zhou, 2004; Chi et al., 2006; Hammer et al., 2006). They are known to play a regulatory role in the production of pro- or anti-inflammatory cytokines following stimulation with LPS, peptidoglycan, or dexamethasone (Hammer et al., 2005; Chi et al., 2006). Together, these reports suggest MKP-1 may play a role in the regulation of inflammation and is a promising possible target for anti-inflammatory drugs.

To gain insight into the role of AR in controlling the immune response and the mechanism by which anti-inflammation is mediated, we have investigated whether, and how, AR is linked to the regulation of anti-inflammation in Raw 264.7 cells and in zymosan air–pouch mice. This study highlights the potential role of AR in anti-inflammation and provides a molecular mechanism for the pharmacological action of AR.

2. Materials and methods

2.1. Preparation of Astragali Radix extracts

A 5-year-old *Astragalus membranaceus* (500 g) sample was purchased from the Korean Association of Crude Medicinal Herbs. The root of *Astragalus membranaceus* (AR) was ground to fine powder and extracted with pure water by sonicating three times for 10 min. The whole supernatant was concentrated at 60 °C under vacuum using an evaporative system (Eyela, Japan) and freeze-dried in a freeze dryer (Eyela, Japan). The dry weight of the extract powder obtained was 66.11 g (yield 13.2%). This AR extract was dissolved in PBS. *Astragalus membranaceus* specimen had been taxonomically identified with respect to morphology by Professor Hocheol Kim, a herbalist at Kyunghee University. The voucher specimen (*Astragalus membranaceus* (Fisch.) Bunge, No. 061110) has been deposited at the Herbarium of College of Oriental Medicine, Kyunghee University, Korea.

2.2. Animals

Female Balb/c mice weighing 19–20 g (12–13 weeks) were housed and cared for under standard conditions, with a 12:12 h day/night cycle, in the Animal Care Facility Service at the Seoul National University (Korea). All experimental procedures utilizing mice were performed in accordance with the National Institute of Health guidelines. The minimum sample size per group, in all animal experiments, was 10.

2.3. Zymosan air–pouch mouse model

An air–pouch was produced by subcutaneously injecting 5 ml of air on the back of each mouse, on the first day. Mice, however, were fed either AR (30 or 100 mg/kg per day) or PBS orally for 7 days prior to induction of the air–pouch. On the fourth day, an additional 5 ml of air was injected into the pouch. Six days after the initial injection of air, 1 ml of either saline or a 1% solution of zymosan (Sigma Chemical Co., St. Louis, MO) was injected into the pouch to induce inflammation. Six days after zymosan injection, the animals were anesthetized with ether and the cavity was flushed out with 2 ml of heparinized (Sigma) saline, and this was repeated twice. The fluid collected from the air–pouches of all members of each group was pooled and cells were separated by centrifugation. Leukocytes and inflammatory cells were recovered and counted in triplicate using a hemocytometer. No significant variations among individuals in each group were verified by a one-way analysis of variance (ANOVA), in all experiments using the pooled exudates collected from air–pouches of all individuals of each group.

2.4. Cell culture and treatment

Murine Raw 264.7 macrophage cells were grown in Dulbecco's modified Eagle's medium (DMEM, GIBCO-Invitrogen, Grand Island, NY) supplemented with 10% FBS (HyClone, Logan, UT). Raw 264.7 cells were grown in six-well plates at a density of $\sim 5 \times 10^5$ cells per well and allowed to grow in DMEM containing 0.1% FBS for 24 h. Cells were untreated or treated with AR (0.1, 0.5, or 1.0 μg/ml) for 30 min before exposure to 100 ng/ml LPS (*Escherichia coli* Serotype 055:B5, Sigma). At the indicated time points following LPS stimulation, cells were harvested.